1997. The Journal of Arachnology 25:222–227

RESEARCH NOTE

ASSESSMENT OF THE RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TECHNIQUE FOR THE ANALYSIS OF SPIDER POPULATIONS

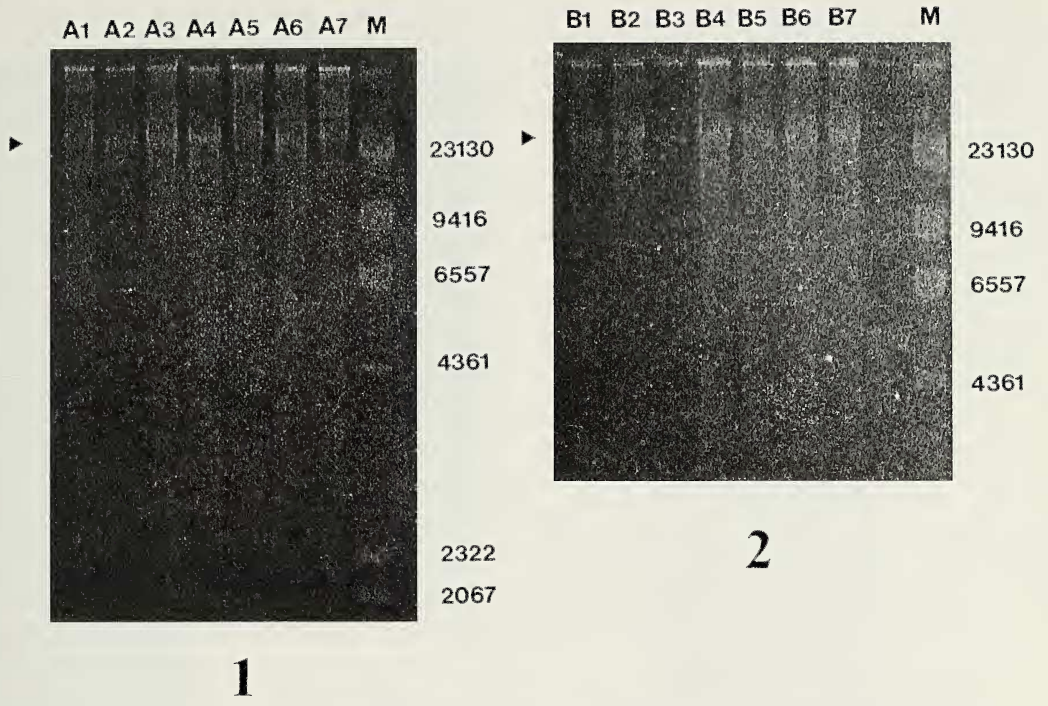
The ability to determine the degree of genetic relatedness between different populations(both geographic and morphologic) of spiders would be of great value in many areas of spider biology. For example, it would allow the testing of the hypotheses that woodland fragments can act as habitat islands (Beaumont 1993) and that spiders can pass freely between real islands by aerial dispersal (Duffey 1956). It would also allow the investigation of the basis (genetic or environmental) of the intra-species size and shape variation seen in geographically separate populations of some species (e.g., Lycosidae: *Trochosa terricola* Thorell 1856 and *Pardosa pullata* (Clerck 1757) from widely separated peat-bog sites (Curtis & Stinglhammer 1986)).

Multi-locus DNA profiling ("finger-printing" Jeffreys et al. 1985a,b, 1991) experiments using the human DNA probes 33.6 and 33.15 (Jeffreys et al. 1985a, b) on genomic DNA of *P. pullata* had previously demonstrated that there were no sequences complementary to either of these probes in the genome of this lycosid (Beaumont 1993). For this reason, and also because spiders (particularly the smaller species) are so small that they might not contain sufficient DNA to generate a conventional DNA profile (5 μg; Bruford et al. 1992), a different approach to genetic analysis was adopted. The technique of random amplified polymorphic DNA (RAPD) analysis (Williams et al. 1990) or DNA amplification fingerprinting (DAF --Caetano-Anolles et al. 1991) has been successfully used in recent years to examine genetic relationships in a wide variety of species, including plants (Virk et al. 1995), insects (Hadrys et al. 1993), humans and other mammals (Welsh et al. 1991; Williams et al. 1990), and micro-organisms (Caetano-Anolles et al. 1991; Welsh & McClelland 1990). This technique uses an arbitrarily chosen oligonucleotide primer in a low stringency polymerase chain reaction (PCR --Saiki et al. 1988; Newton & Graham 1994) to generate a series of amplified products of different sizes from a target (usually intact genomic) DNA. These products are then separated by conventional agarose or polyacrylamide gel electrophoresis (Sambrook et al. 1990). The pattern of fragments produced is specific for the combination of primer and target DNA used (and the precise conditions of the PCR) and is attributed to the distribution of primer-complementary sequences within the target DNA. Since this is an amplification method, it can generate useful data from very small amounts of target DNA (e.g., as little as 1 ng; Hedrick 1992). Also, because of the ease of synthesis of primers and the speed of the PCR itself, it is feasible to screen large numbers of primers under different experimental conditions until a combination producing a suitable pattern of bands is found.

A preliminary investigation was thus undertaken to assess the suitability of the RAPD method for the genetic analysis of spider populations. The study used six different primers and genomic DNA isolated from siblings of two different broods of the theraphosid *Brachypelma albopilosa* (Valerio 1980). This spider was chosen because it was possible to purchase broods of known parentage. It was hoped to identify one or more primer(s) that would reliably enable both identification of siblings and discrimination between members of different broods.

METHODS

Two broods of juvenile (third instar) specimens of *B. albopilosa* of different parentage



Figures 1, 2.—Agarose gels (0.8%) showing intact, high molecular weight genomic DNA(►) isolated from specimens of *Brachypelma albopilosa* from two different broods: 1, brood A; 2, brood B. 1–7 are different individuals within each brood. Each sample represents 10% of the total amount of DNA isolated from each specimen. M = marker DNA: genomic DNA of bacteriophage λ digested with the restriction enzyme *Hin*dIII. Sizes of the DNA fragments are indicated in base pairs (bp.). There is 0.5 μg of DNA in the 23,130 bp. band. The amount of DNA in each sample of *Brachypelma albopilosa* DNA was estimated by comparison with the bands in the marker lane as recommended by Bruford et al. (1992).

were purchased from Ronald N. Baxter (Entomological Supplies),45 Chudleigh Crescent, Ilford, Essex, IG3 9AT, England, UK. These were designated A and B, maintained under starvation conditions for four days after delivery (to ensure ablation of intestinal fauna) and then stored at −80 °C. Voucher material of these specimens has been deposited at the Paisley Natural History Museum, Paisley Museum & Art Galleries, High Street, Paisley, PA1 2BA, Scotland, UK. DNA was extracted from these whole, frozen, individual spiders using a protocol based on a procedure for the isolation of intact, high molecular weight DNA from mammalian cells described by Sambrook et al. (1990) and described in full elsewhere (Beaumont 1993). After extraction, an aliquot of each DNA sample was run on an agarose gel to assess concentration, purity and integrity.

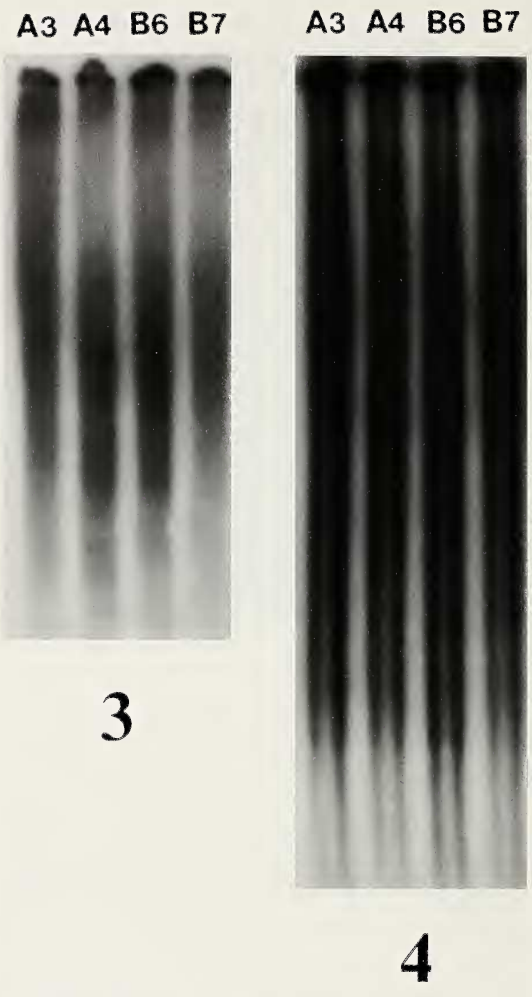
Six oligonucleotide primers were synthesized for use in these experiments. Some of the sequences were chosen because of their usefulness in previous RAPD experiments (e.g., 1 & 4 by Caetano-Anolles et al. 1991); others were selected as they had been successfully used as probes in conventional DNA profiling experiments (2 & 3 by Weising et al. 1989; 2 by Debarro et al. 1994); still others were wholly arbitrary (5 & 6). The sequences of these primers (shown 5′–3′ from left to right) are as follows: primer 1: CGCGCCGG, primer 2: GATAGATAGATAGATA, primer 3: GACAGACAGACAGACA, primer 4: GTGATCGCAG, primer 5: GTAAAACGACGGCCAGT, primer 6: CTAGGTCTTGAAAGGAGTGC.

Polymerase chain reactions, using 16 ng of *B. albopilosa* genomic DNA as target, were performed as described (Welsh et al. 1991), except that the annealing temperatures were kept constant throughout all the

cycles of any one reaction. Two sets of reactions were carried out for each primer, the first with an annealing temperature of 40 °C, the second with an annealing temperature of 30 °C. Agarose and polyacrylamide gels were run as described in Tris-borate electrophoresis (TBE) buffer (Sambrook et al. 1990). Agarose gels (0.8%) were used to assess the concentration, purity and integrity of intact genomic DNA; 5% polyacrylamide gels (± 7M urea) were used to resolve the products of the PCR reactions. If appropriate, gels were stained in a solution of ethidium bromide (0.5 $\mu g ml^{-1}$ in TBE) for 15–30 min. Polyacrylamide gels were dried under vacuum at 80 °C (2 h), wrapped in plastic film ("Saran-Wrap", Dow Chemical Co.) and any bands visualized by exposure of the gel to autoradiography film ("Hyperfilm-MP", Amersham, UK) in an autoradiography cassette fitted with two intensifying screens for 1–4 days at −80 °C.

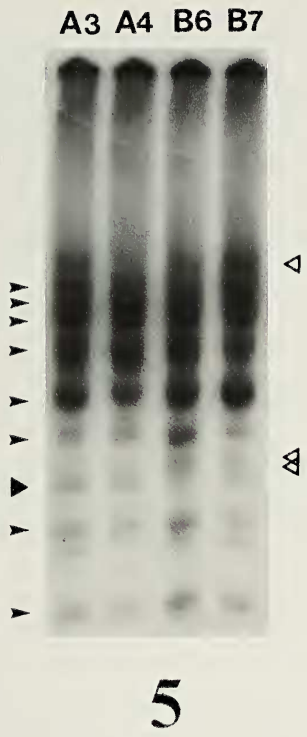
RESULTS AND DISCUSSION

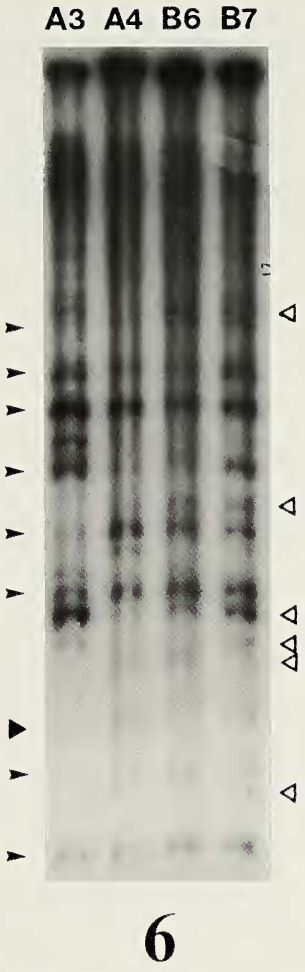
Intact, high molecular weight genomic DNA was successfully and consistently isolated from *B. albopilosa* (Figs. 1, 2). The amount of DNA isolated from each spider was estimated to be 0.25–1.0 μg, sufficient for many RAPD analyses to be performed on each individual. In other studies, intact, high molecular weight genomic DNA was successfully isolated from other spider species (Amaurobiidae: *Amaurobius similis* (Blackwall 1845); Linyphiidae: *Lepthyphantes leprosus* (Ohlert 1865); Lycosidae: *Pardosa pullata;* Araneidae: *Zygiella x-notata* (Clerck 1757)) by the same method (not shown). The genomic DNAs from two individuals from each brood (3 & 4 from A, 6 & 7 from B) were used in polymerase chain reactions with each of the six primers (separately). The reaction products were separated by polyacrylamide gel electrophoresis, the gels dried and the DNA fragments visualized by autoradiography. The results of these reactions are summarized in Tables 1–6, and examples of the banding patterns produced are shown in Figs. 3–6. It should be noted that the DNA fragments on several gels were also visualized by ethidium bromide staining before drying (not shown) and a comparison of the stained gel and autoradiograph showed that no additional bands were detected by autoradiography. Thus, it is unnecessary to use radio-labelled nucleotides in the RAPD procedure, thus simplifying this process.



Figures 3, 4.—Autoradiographs showing the results of RAPD analysis on individuals A3, A4, B6 and B7 using primer 3 with a PCR annealing temperature of: 3, 40°C; 4, 30 °C.

The only primer that consistently failed to produce amplified products of discrete sizes was primer 3 (Figs. 3, 4). At both annealing temperatures, this primer generated many fragments of similar sizes that were not resolved by the electrophoretic technique used, resulting in the appearance of long smears on the autoradiographs. This suggests that there are many binding sites complementary to this primer throughout the tar-





Figures 5, 6.—Autoradiographs showing the results of RAPD analysis on individuals A3, A4, B6 and B7 using primer 4 with a PCR annealing temperature of: 5, 40 °C; 6, 30 °C. Products common to all four samples (➤), or to A3 and A4 only (►), or to B6 and B7 only (◁) are indicated.

get DNA. It is possible that this primer is annealing to microsatellite loci and that annealing is occurring at many overlapping sites. For all the other primers used, products of discrete sizes (different in both number and size for each primer) were consistently produced. This suggests there are smaller numbers of complementary sites available to which these primers can anneal. The exact pattern produced by a primer var-

Table 1.—Total number of DNA fragments generated by PCR amplification from each target DNA at each annealing temperature by primers 1, 2, 4–6.

	Primer 1		Primer 2		Primer 4		Primer 5		Primer 6	
DNA	30 °C	40 °C	30 °C	40 °C	30 °C	40 °C	30 °C	40 °C	30 °C	40 °C
A3	2	5	5	2	15	11	5	9	8	7
A4	4	5	6	6	11	9	8	9	5	6
B6	8	5	7	8	15	11	6	13	5	6
B7	7	3	5	7	16	12	4	11	5	4

Table 2.—Number of common amplified DNA fragments generated from target DNAs A3 and A4 at each annealing temperature by primers 1, 2, 4–6.

Primer	Annealing temperature	
	30 °C	40 °C
1	2	3
2	5	0
4	9	9
5	5	9
6	2	6

Table 3.—Numbers of common amplified DNA fragments expressed as percentages of total number of DNA fragments generated from target DNAs A3 and (A4) at each annealing temperature by primers 1, 2, 4–6.

Primer	Annealing temperature	
	30 °C	40 °C
1	100 (50)	60 (60)
2	100 (83)	0 (0)
4	60 (82)	82 (100)
5	100 (63)	100 (100)
6	25 (40)	86 (100)

ied according to the annealing temperature used during the PCR. Primer 4 provides good examples of the type of pattern produced (Figs. 5, 6). At both annealing temperatures, this primer generated many amplified products from each target DNA and the patterns of products observed were very similar from all four target DNAs. Due to these similarities, reliable differentiation between inter- and intra-family relationships was not possible using this primer. And, indeed, for this same reason, none of the primers used could reliably differentiate between these types of relationships (see Tables 1–6).

Table 5.—Numbers of common amplified DNA fragments expressed as percentages of total number of DNA fragments generated from target DNAs B6 and (B7) at each annealing temperature by primers 1, 2, 4–6.

Primer	Annealing temperature	
	30 °C	40 °C
1	50 (57)	60 (100)
2	57 (80)	50 (57)
4	93 (88)	100 (92)
5	67 (100)	77 (91)
6	100 (100)	67 (100)

Although no primer was found that could allow both identification of siblings and discrimination between members of different broods, the feasibility of the procedure was established. Analysis of the organization and sequence of the *B. albopilosa* genome, particularly of any repetitive sequences that may be found, should permit the design of more useful primers. It should be noted, however, that there are known to be some problems associated with RAPD analysis. For example, and as in all DNA profiling analyses, it can be difficult to determine whether similar bands in different lanes are matching or not. Also, distinguishing heterozygotes from homozygotes and establishing Mendelian patterns of inheritance may prove problematic. Finally, there can be problems of data reproducibility between experiments. Some of these problems, and methods that might be employed to over-

Table 4.—Number of common amplified DNA fragments generated from target DNAs B6 and B7 at each annealing temperature by primers 1, 2, 4–6.

Primer	Annealing temperature	
	30 °C	40 °C
1	4	3
2	4	4
4	14	11
5	4	10
6	5	4

Table 6.—Numbers of common amplified DNA fragments generated at each annealing temperature from target DNAs A3, A4, B6 and B7 by primers 1, 2, 4–6.

Primer	Annealing temperature	
	30 °C	40 °C
1	0	3
2	3	0
4	8	8
5	4	6
6	2	3

come them, are discussed by Williams et al. (1990) and Hedrick (1992).

We are grateful to C.A. Hopkins, D.S.T. Nicholl and J.R.M. Thacker for their comments on the manuscript.

LITERATURE CITED

Beaumont, D.J. 1993. Community ecology studies on spiders of island habitats in western Scotland. Ph.D. thesis, University of Paisley, Paisley, Scotland. 237 pp.

Bruford, M.W., O. Hanotte, J.F.Y. Brookfield & T. Burke. 1992. Single-locus and multilocus DNA fingerprinting. Pp.225–269, *In* Molecular Genetic Analysis of Populations —A Practical Approach (A.R. Hoelzel, ed.) IRL Press, Oxford.

Caetano-Anolles, G., B.J. Bassam & P. Gresshoff. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Biotechnology, 9:553–557.

Curtis, D.J. & H.R.G. Stinglhammer. 1986. Wanderers and webspinners on Scottish peatbogs: size variations. Actas X Congr. Int. Arachnol. Jaca/España, I:219–223.

Debarro, P., T. Sherratt, S. Wratten & N. MacLean. 1994. DNA fingerprinting of cereal aphids using $(GATA)_4$. European J. Entomol., 91:109–114.

Duffey, E. 1956. Aerial dispersal in a known spider population. J. Anim. Ecol., 25:85–111.

Hadrys, H., B. Schierwater, S.L. Dellaporta, R. Desalle & L.W. Buss. 1993. Determination of paternity in dragonflies by random amplified DNA fingerprinting. Molec. Ecol., 2:79–87.

Hedrick, P. 1992. Shooting the RAPDs. Nature, 355:679–680.

Jeffreys, A.J., A. MacLeod, K. Tamaki, D.L. Neil & D.G. Monckton. 1991. Minisatellite repeat coding as a digital approach to DNA typing. Nature, 354:204—209.

Jeffreys, A.J., V. Wilson & S.L. Thein. 1985a. Hypervariable "minisatellite" regions in human DNA. Nature, 314:67–73.

Jeffreys, A.J., V. Wilson & S.L. Thein. 1985b. Individual specific "fingerprints" of human DNA. Nature, 315:76–79.

Newton, C.R. & A. Graham. 1994. PCR—Polymerase Chain Reaction. Bios Scientific Publ., Oxford.

Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis & H.A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with athermostable DNA polymerase. Science, 239:487–491.

Sambrook, J., E.F. Fritsch & T. Maniatis. 1990. Molecular Cloning —A Laboratory Manual (2nd ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Virk, P.S., B.V. Ford-Lloyd, M.T. Jackson & H.J. Newbury. 1995. Use of RAPD for the study of diversity within plant germplasm collections. Heredity, 74:170—179.

Weising, K., F. Weigand, A.J. Driesel, G. Kahl, H. Zischler & J.T. Epplen. 1989. Polymorphic simple GATA/GACA repeats in plant genomes. Nucl. Acids Res., 17:10128.

Welsh, J. & M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res., 18:7213–7218.

Welsh, J., C. Petersen & M. McClelland. 1991. Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. Nucl. Acids Res., 19:303–306.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski & S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., 18:6531–6535.

Simon J.H. Hettle, Wendy Hall, Susan L. Dyas, David J. Beaumont, Dahlia M. Caplan & David J. Curtis: Dept. of Biological Sciences, University of Paisley, Paisley PA1 2BE, Scotland, UK.

Manuscript received 7 December 1995, revised 10 October 1996.